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Aural computed tomography characteristics of American cocker spaniels have features of both mesaticephalic and brachycephalic breeds.

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Abbreviations:

TA: transverse area

TA1: transverse area of the osseous horizontal ear canal

TA2: transverse area of the widest air-filled part of the cartilaginous horizontal ear canal

ABSTRACT

Otitis externa and otitis media are common in American cocker spaniels, however breed-specific aural computed tomography (CT) descriptions are currently lacking. This prospective and retrospective, observational, analytical study aimed to describe quantitative CT characteristics of the horizontal ear canal and the tympanic bulla in American cocker spaniels versus similar-sized mesaticephalic dogs. We prospectively performed clinical examinations and aural CT scanning for 38 American cocker spaniels. Computed tomographic transverse area of the osseous (TA1), the widest air-filled part of the cartilaginous (TA2) horizontal ear canal, tympanic bulla volume and tympanic bulla wall thickness were measured. The TA1 and TA2 measurements were compared with those of 23 retrospectively recruited, similar-sized mesaticephalic dogs that had undergone CT-scanning of the head for non-ear related reasons. No significant differences appeared in TA1 and TA2 between healthy American cocker spaniels and mesaticephalic dogs, but severely affected American cocker spaniels had significantly smaller TA2 (P<0.001). The intraclass correlation coefficient for intra-observer and inter-observer repeatability was 0.972 and 0.983 for TA1 and 0.994 and 0.998 for TA2, respectively. Variation between individuals was subjectively noted in healthy and affected American cocker spaniels, but the mean tympanic bulla volume was slightly smaller in relation to the body weight, and the tympanic bulla wall was thicker than in previous reports for mesaticephalic dogs. The tympanic bulla wall appeared thicker rostro-ventrally than caudo-ventrally in 44% of the dogs. Our results imply that a relatively thick tympanic bulla wall may be a normal CT variation and should be interpreted cautiously in this breed.

INTRODUCTION

Cocker spaniels are reported to be overrepresented among breeds that suffer from otitis externa,[1-4] one of the most common dermatological conditions in dogs.[5] In a recent survey, the prevalence of otitis externa in American cocker spaniels was 27%.[6] Accordingly, they are also overrepresented among breeds requiring total ear canal and bulla osteotomy surgery as a treatment for chronic otitis externa.[7-10]

Otitis media accompanies chronic otitis externa in up to 80% of the cases.[11] However, the diagnosis of otitis media is often difficult, and diagnostic imaging can be used to aid the diagnosis.[12] Computed tomography (CT) and magnetic resonance imaging (MRI) are more sensitive than traditional radiography or ultrasonography in diagnosing otitis media.[13-18] Currently, CT is considered the gold standard method to evaluate the tympanic bulla.[19,20] While CT is becoming increasingly available in veterinary practice, it is often used to evaluate the pathological changes caused by chronic otitis, especially when assessing whether surgery is required.[21]

CT anatomy of the ear has been described in dogs.[22] Anatomical comparisons of the tympanic bulla volume and wall, as well as tympanic bulla shape and position, have been described in mesaticephalic and brachycephalic dogs.[23-25] Previous studies have shown that brachycephalic breeds have a smaller tympanic bulla volume and a thicker bulla wall than mesaticephalic breeds, and that there is interbreed variation in the shape and position of the tympanic bulla. For example, Cavalier King Charles spaniels appear to have a flattened tympanic bulla,[25] and English bulldogs have a narrower horizontal ear canal than mesaticephalic breeds.[26] However, breed-specific aural CT measurements are lacking in non-brachycephalic breeds with high-risk for otitis externa, such as American cocker spaniels, but would be beneficial in the diagnostics of ear diseases in these breeds.

The present study aimed to describe the anatomical CT-characteristics of the horizontal ear canal and the tympanic bulla in American cocker spaniels with healthy and diseased ear canals. Another aim was to evaluate differences in the transverse area (TA) measurements of the horizontal ear canal between healthy American cocker spaniels and other mesaticephalic dog breeds, and among American cocker spaniels of different ear-health conditions. In addition, this study aimed to investigate the intra-observer and inter-observer repeatability of the TA measurements. Our hypothesis was that the horizontal ear canal in American cocker spaniels is narrower than in a group of similar-sized mesaticephalic dogs and that the repeatability of the measurements within and between observers is high.

MATERIALS AND METHODS

Case selection

This study was a prospective and retrospective, observational, analytical design. All procedures were approved by the Finnish National Animal Experiment Board (ESAVI-1662/04.10.03/2011). The prospective part of the study was carried out at the Veterinary Teaching Hospital of the University of Helsinki during the period of march to september 2011. All owners signed an informed consent before entering the study. One of the authors (a senior veterinary clinician, M.K.) evaluated each participating dog to match the inclusion criteria: pure-bred American cocker spaniel, aged at least 12 months with or without a history of otitis externa, and no known conditions that increase the risk of complications from anesthesia. All animals meeting inclusion criteria during a 6-month study recruitment period were included in the study.

Clinical examination

Clinical examination of all the dogs was performed by a licensed veterinarian (M.K.). The dogs were sedated intramuscularly with medetomidine 10 ug/kg (Domitor®, Orion Pharma, Espoo, Finland) and butorphanol 1 mg/kg (Torbugesic®, Forte-Dodge, Iowa, USA), and their ears were examined with a video-otoscope (Dr Fritz GmbH, Tuttlingen, Germany). The presence or absence of otitis externa was based on the OTIS3 score as described by Nuttal et al. (2014).[27] A score between 0–12 was assigned to each ear, and a total ear score of ≥ 4 differentiated affected from clinically healthy ears. In addition, samples for cytology were taken from each ear. Cytology slides were prepared and examined under oil immersion (OI, x 1000) as described previously.[4]

Computed tomography imaging

Following clinical examination, CT-scanning was performed on the ears using a helical dual slice scanner (Somatom Emotion Duo; Siemens AG, Forchheim, Germany) with 130 kVp, 113 mA, with a bone algorithm. The slice thickness was 1 mm, feed/rotation 2 mm, and reconstruction increment 0.5 mm. The dogs were placed in a sternal recumbency and their head was supported straight. They received midazolam 0.1 mg/kg IV (Midazolam Hameln, Siegfried Hameln GmbH, Hameln, Germany) if more sedation was needed. The images were stored in DICOM format in the hospital's PACS until analysis.

To compare the horizontal ear canal measurements between American cocker spaniels and mesaticephalic dogs, the database of Veterinary Teaching Hospital of University of Helsinki was searched for dogs that matched the prospectively sampled animals as much as possible and had undergone CT-scanning of the head for reasons unrelated to ear disease. The inclusion criteria for these dogs were: mesaticephalic breed weighing 5–20 kg, aged at

least one year. Dogs were excluded if there was any history or clinical findings of ear or skin disease in the database, if the slice thickness of the scan was more than 2mm, or if there were incidental abnormalities (for example otolithiasis) in the CT images. A veterinary radiology specialist (A.K.L., 15 years of experience in veterinary radiology) reviewed the CT images of all retrieved cases to ensure that the inclusion criteria were fulfilled.

Image analysis

Image evaluations were performed by the veterinary radiology specialist (A.K.L.) and two licensed veterinarians (observer A: M.K., and observer B: V.R.). All CT studies were viewed using commercially available software (OsiriX MD, version 9.0, OsiriX Foundation, Geneva, Switzerland). In American cocker spaniels, the following parameters were recorded from the CT images: 1) transverse surface area (mm^2) of the osseous part of the horizontal ear canal (TA1), 2) transverse surface area (mm^2) of the widest air-filled part of the cartilaginous horizontal ear canal (TA2), 3) thickness (mm) of the tympanic bulla wall, 4) tympanic bulla volume (mm^3), 5) presence of fluid/soft tissue material in the tympanic bulla, subjectively ranked as mild (<30%), moderate (30–60%) or marked (>60%), 6) presence of ear canal mineralization, subjectively ranked as mild (<30%), moderate (30–60%) or marked (>60%), 7) skull width (cm), and 8) skull height (cm). In mesaticephalic dogs, the horizontal ear canal measurements (TA1 and TA2), and the skull measurements (width and height) were performed.

The veterinary radiology specialist selected multiplanar reconstruction images for the measurements of the TA (mm^2) of the horizontal ear canal. The window level and width were 300 HU and 1500 HU, respectively, following Seppänen et al. (2019).[26] First, the

dorsal image plane was determined from the midsagittal image as a parallel plane to the skull base (Figure 1A). Second, the transverse image was straightened at the level of the middle ears using the cochleae as landmarks. The parasagittal image for measuring the TA of the osseous ear canal (TA1) was the one in which the bony wall was most clearly seen (Figure 1B). The oblique parasagittal image for measuring the TA of the widest air-filled part of the cartilaginous horizontal ear canal (TA2) was selected as the one in which the cross-section of the canal was considered the largest (Figure 1C). The images were randomized, and the observers were blinded to the background data of the dogs. Before all measurements, observers A and B familiarized themselves with the measurement techniques using practice images under the supervision of the veterinary radiology specialist. The TA was determined by manually drawing a region of interest with the software's pencil tool, tracing the margin of the bony wall of the osseous (TA1) and the widest air-filled (TA2) cartilaginous horizontal ear canal (Figure 2). Observers A and B performed these measurements twice, one week apart, for intra-observer and inter-observer repeatability testing. Finally, the mean of the four TA1 and TA2 measurements (mm^2) in each dog was calculated and used for the study.

Observer A measured the thickness of the tympanic bulla wall. It was measured at its most ventral point parallel to the midline in the transverse plane images (middle-ear algorithm, window level 700 HU, window width 4000 HU) at two levels – at the level of the tympanic membrane (caudo-ventral aspect) and at the level of the cochlea (rostro-ventral aspect) (Figure 3). Observer B measured the tympanic bulla volume using the same image parameters. It was measured by calculating all the sequential hand-drawn regions of interest together using the computer program. The internal surface of the osseous bulla including

the three middle ear bones was manually traced in all slices. In the sections located at the level of the tympanum and where the tympanic membrane was not visible, a straight line was traced artificially to connect the medial edges of the horizontal ear canal by using a 45-degree angle in relation to the central axis of the canal. After tracing the region of interest in all slices of each ear, the total volume of each middle ear was calculated automatically by the software. In addition, to compare the horizontal ear canal measurements with the skull size, observer A measured the skull width and height from the transverse image of the skull at the same multiplanar reconstruction level and window settings as the tympanic bulla caudo-ventral measurements. The skull width was measured as the widest part of the skull dorsal to the cochleae in the horizontal plane; the skull height was the measurement from the base of the skull to the top of the skull in the midline (Figure 4). The skull width and height were calculated together to describe the skull size.

Statistical analysis

The American cocker spaniels were divided into four groups according to the history of otitis externa and the total ear score: clinically healthy ears (score 0–3 in both ears) with no history of ear disease (group 1), clinically healthy ears (score 0–3 in both ears) with a history of ear disease (group 2), mild otitis externa (score 4–5 in either or both ears, group 3) and severe otitis externa (score ≥ 6 in either or both ears, group 4).

A statistician (S.M.) performed all statistical analyses using statistics software (SAS® System for Windows, version 9.4, SAS Institute Inc., Cary, NC, USA). For investigating differences in TA1 and TA2 between healthy American cocker spaniels (group 1) and mesaticephalic dogs, a random effects ANCOVA model was fitted with a fixed effect of breed group, covariates of age, weight, gender, and skull sum, and a random effect of dog. TA1,

TA2, the tympanic bulla wall thickness (rostral and caudal), and the tympanic bulla volume were also compared between the four American cocker spaniel groups. These endpoints were modelled as described above, except the American cocker spaniel group was included in the model instead of the breed group. In addition, in American cocker spaniels, associations between horizontal ear canal edema or exudate and TA2-values were investigated with one-way ANOVA models. Associations between different aural cytological findings or the presence of ear canal mineralization and the presence of tympanic bulla effusion were assessed with mixed effects logistic regression models. Dog was used as a random effect in both, the ANOVA and the logistic regression models.

In the TA1 and TA2 measurements, the intraclass correlation coefficient (ICC) was calculated both for intra-observer and inter-observer comparisons. For inter-observer calculations the mean of the two measurements was used for each assessor. In addition to ICC, a random effects model was fitted separately for both comparisons to estimate the variance components related to repeating the assessment by the same observer (intra-observer comparison), or the variance component related to the observer (inter-observer comparison). Both models included dog as a random effect. Values of ICC 0.01–0.2 were considered to have "slight agreement", 0.21–0.40 "fair agreement", 0.41–0.60 "moderate agreement", 0.61–0.80 "substantial agreement", and 0.80–1.00 "almost perfect agreement".[28] Intra-observer and inter-observer coefficients of variation for TA1 and TA2 were calculated using the root mean square method.

For ANCOVA models, estimates of group differences and within-group values were calculated from the fitted models with contrasts. For the group differences and within-group values, 95 % confidence intervals (CI) and *P*-values were calculated. For the multiple comparisons between American cocker spaniel groups, the *P*-values were adjusted with the

Tukey-Kramer method. Age, weight, and skull sum were tested with T-tests for comparison between healthy American cocker spaniels and mesaticephalic dogs. For comparisons among the American cocker spaniel groups, a one-way ANOVA was used for these variables. Differences between groups in the proportion of each gender were estimated with Fisher's exact test. Normality assumptions for parametric models were confirmed with Kolmogorov-Smirnov tests, for borderline cases also the skewness parameter was assessed. *P*-values < 0.05 were considered statistically significant.

Additionally, it was evaluated whether the measured tympanic bulla volume followed the previously published equation for middle ear canal volume (MECV) = -0.612 + 0.757 * ln (bodyweight).[23] The MECV values were calculated for all dogs based on the equation, and compared with the measured tympanic bulla volume values. To describe the difference, a Bland-Altman plot was created. Bland-Altman plot presented the difference between MECV and measured tympanic bulla volume values against the mean of these two.

RESULTS

Study population

Thirty-eight (26 females, 12 males) privately owned American cocker spaniels met the inclusion criteria and were enrolled in the study. The database search found 23 dogs (12 females, 11 males) representing 18 different breeds to be included in the retrospective part of the study. Most of these dogs (n = 21) had been imaged with the same scanner as the American cocker spaniels in this study; the remaining two were scanned with a helical 64-slice scanner (Lightspeed VCT, GE Healthcare, Wisconsin, USA).

Clinical examination findings

During the clinical examination, 18 of 38 (47%) American cocker spaniels were considered clinically healthy in both ears and were assigned to either group 1 (8 females, 1 male) or to group 2 (4 females, 5 males). Group 3 comprised seven (6 females, 1 male) dogs with unilateral (n = 1) or bilateral (n = 6) mild otitis externa, and group 4, 13 (8 females, 5 males) dogs with unilateral (n = 4) or bilateral (n = 9) severe otitis externa. One dog with unilateral severe otitis externa had earlier total ear canal ablation and bulla osteotomy surgery performed on the other ear, so a total of 75 ears were examined. OTIS3 scoring and aural cytology results are shown in Table 1.

Measurements of the horizontal ear canal

In American cocker spaniels´ group 1 and in mesaticephalic dogs, the mean TA1 was 27.2 mm^2 and 29.2 mm^2, whereas the mean TA2 was 37.1 mm^2 and 35.6 mm^2, respectively. TA1 and TA2 showed no significant differences between these two groups (Table 2). Significant differences were detected in age ($P = 0.042$) and gender ($P = 0.050$).

In American cocker spaniels (groups 1–4), the mean TA1 showed no significant differences between the four groups (Figure 5). In the analysis of TA2, a significant group effect was found ($P < 0.001$). TA2 values were significantly lower in group 4 than in the other groups. (Figure 6). Marked edema ($P < 0.001$) and moderate or marked amount of exudate ($P < 0.001$) in the horizontal ear canal all significantly diminished TA2.

Repeatability in TA1 and TA2 measurements showed almost perfect agreement within and between observers. For observer A, ICC was 0.959 for TA1 and 0.990 for TA2; for observer B, ICC was 0.985 for TA1 and 0.998 for TA2. Overall ICC-values for the intra-observer and inter-observer repeatability were 0.972 and 0.983 for TA1, and 0.994 and 0.998 for TA2, respectively. Intra-observer and inter-observer coefficients of variation were 3.2% and 2.3% for TA1 and 3.7% and 2.0% for TA2, respectively.

257 Ear canal mineralization was observed in 22 of 75 ears (29%). None of 18 ears
258 in group 1, 1/18 (6%) ears in group 2, 4/14 (29%) ears in group 3 and 17/25 (68%) ears in
259 group 4 had evidence of ear canal mineralization. The mineralization was considered mild in
260 16 ears (groups 2–4), moderate in 2 (group 4), and severe in 4 (group 4) ears.
261 Measurements of the tympanic bulla
262 In group 1, the mean tympanic bulla volume was 0.77 mm^3 and the tympanic
263 bulla wall thickness varied between 0.7 mm and 2.7 mm (Table 3, Figures 3 and 7). The mean
264 thickness of the tympanic bulla wall was 1.3 mm at the level of the tympanic membrane, and
265 1.6 mm at the level of the cochlea. In 33 of 75 (44%) ears (groups 1-4), the thickness of the
266 bulla wall was at least 0.3 mm thicker at the rostro-ventral measurement point. Groups 1–4
267 showed no significant differences in the tympanic values (Table 3). The Bland-Altman plot
268 showed that the overall mean difference detected between the calculated MECV and the
269 measured tympanic bulla volume was 0.34 mm^3 (Figure 8).
270 Six American cocker spaniels had fluid or soft tissue material in their bulla
271 either bilaterally (n = 5) or unilaterally (n = 1). The amount of material in the bulla was
272 considered mild in 2, moderate in 3 and marked in 6 ears. Nine out of 22 ears (41%) with
273 clinically severe otitis externa showed material in the bulla. One dog with clinically mild otitis
274 externa in the left ear had a marked amount of material in the left bulla and a mild amount
275 of material in the right bulla; the right external ear was considered clinically healthy. The
276 presence of ear canal mineralization (P = 0.016), as well as the presence of cocci (P = 0.031)
277 or rod-shaped bacteria (P = 0.026) in cytology of the aural exudate, were associated with the
278 presence of bulla effusion.
279
280 DISCUSSION

281 This study was intended to provide quantitative and comparative CT data for
282 the horizontal ear canal and the tympanic bulla in American cocker spaniels, a high-risk
283 breed for otitis externa. Findings indicated that the transverse area of both the osseous and
284 the cartilaginous part of the horizontal ear canal in clinically healthy American cocker
285 spaniels did not differ from mesaticephalic dogs, which was contradictory to our hypothesis.
286 It appears that, in comparison to other similarly sized breeds, an anatomically narrower
287 horizontal ear canal is not the explanation why otitis externa and end-stage ear disease are
288 overrepresented in American cocker spaniels. However, the tympanic bulla wall was thicker
289 and the tympanic bulla volume smaller in this breed than what has been previously
290 published for mesaticephalic dogs. These results indicated that breed-specific data may be
291 important when interpreting aural CT images.
292 Previous studies have shown that body weight correlates positively with the
293 overall length and diameter of the distal opening of the ear canal.[29] Similarly, the volume of
294 the ear canal is dependent on body weight.[30] However, anatomic conformation of the skull
295 also affects aural morphology.[23-26] Our results showed that the size of the horizontal ear
296 canal lumen was similar in all the examined American cocker spaniels and mesaticephalic
297 dogs, with the mean TA of the osseous part of the horizontal ear canal being less than 30
298 mm^2. Taking into account the slight difference in methods, our measurement results of the
299 horizontal ear canal are in line with previous studies.[26,29] Interestingly, a small and narrow
300 horizontal ear canal does not necessarily cause otitis externa, as most of the English bulldogs
301 with very narrow horizontal ear canals did not suffer from otitis.[26] However, with
302 proliferative changes of the ear canal, the small lumen may readily disappear completely.
303 Compared to other breeds, American cocker spaniels present with ceruminous gland
304 hyperplasia and ectasia more often, which may lead to anatomical changes of the ear canal

and occlusion of the ear canal lumen, resulting in end-stage otitis.[10] Mineralization of the ear canal was very common in severely affected ears in this study, but previous studies have reported mild ear canal mineralization to be common even in clinically healthy dogs.[31] Notably, severe ear canal mineralization was ten times more common in our study than in the previous study. [31]

We also evaluated the repeatability of the TA measurements within and between observers. The method showed almost perfect agreement in the intra- and inter-observer measurements. The variance components showed that the variation between dogs was much larger than the variation between repeats and assessors. To further diminish the impact of human measurement error in the future, automated measurement techniques could be developed.

In American cocker spaniels, the tympanic bulla wall thickness varied substantially between individuals within groups, including groups 1 and 2. However, the mean tympanic bulla wall thickness at the level of the tympanic membrane was 30% thicker than what is previously reported for mesaticephalic dog breeds.[24] Moreover, in the previous study, [24] the mean rostro-ventral measurements were similar to the caudo-ventral measurements in mesaticephalic dogs. In our study, the tympanic bulla wall appeared thicker rostro-ventrally in 44% of the American cocker spaniels. A thicker tympanic bulla wall rostro-ventrally is typical for brachycephalic breeds.[24] In our study, the difference was not related to the positioning of the dogs, as all studied dogs were positioned in the same way. In addition, the finding was bilateral – the left and the right bulla showed the same result in clinically healthy American cocker spaniels. These results imply that the tympanic bulla wall may be relatively thick also in some non-brachycephalic breeds due to anatomical variations in the conformation. Moreover, the mean rostro-ventral measurement showed a thicker

329 tympanic bulla wall at this level in both healthy and diseased American cocker spaniels,
330 indicating that the tympanic bulla anatomy in this breed is slightly different from
331 mesaticephalic breeds. Thus, in the absence of other abnormalities, a bilaterally symmetric
332 thick bulla wall should not be diagnosed as otitis media in this breed, as it may be a normal
333 variation.

334 The tympanic bulla volume also showed variation between individuals. The
335 mean tympanic bulla volume (0.84 mm^3) was smaller than what is reported for
336 mesaticephalic breeds in general (1.50 mm^3).[23] In relation to the body weight of the dog, the
337 tympanic bulla volume did not follow the previously reported MECV equation[23] very well.
338 The study of Defalque et al. (2005)[23] included only a few middle-sized (10 to 20kg) dogs,
339 which could explain the difference. Overall, the MECV equation seemed to give larger values
340 compared to the measured tympanic bulla volume. However, the bulla volume was in line
341 with the value previously reported for mesaticephalic dogs weighing less than 15 kg.[24] Our
342 results indicate that in American cocker spaniels the tympanic bulla volume is smaller in
343 relation to the body weight than what is reported previously in mesaticephalic dogs, but
344 there is variation between individuals. However, further studies with a larger number of
345 dogs are required to verify our results and to evaluate whether this anatomical variation has
346 any impact on the incidence of otitis in this breed.

347 Effusion in the bulla indicating likely otitis media was noted in 11 ears, the
348 majority in ears with clinically severe otitis externa. Mineralization of the ear canal and the
349 presence of cocci or rod-shaped bacteria in aural cytology were associated with tympanic
350 bulla abnormalities, while suppurative otitis was not, unlike in the study of Belmudes et al.
351 (2018).[32] Differences in study populations are likely to explain these discrepancies. The study
352 of Belmudes et al. comprised dogs with chronic otitis externa, while majority of dogs in our

353 study had clinically healthy ears. Middle ear effusion may also appear in dogs without a
354 history or clinical findings of ear disease, especially in brachycephalic dog breeds.[18,24-25] In
355 such cases, the effusion may reflect accumulation of fluid unrelated to inflammation.[18] For
356 example trigeminal nerve disorder or mass may cause denervation of the tensor veli palatini
357 muscle and dysfunction of tuba auditiva, leading to middle ear effusion.[33,34] In one dog, the
358 presence of bilateral bulla effusion was a surprising finding, because one ear showed only
359 mild otitis externa and the other ear was considered clinically healthy, underlining the
360 importance of diagnostic imaging in diagnosing middle ear abnormalities.

361 This study has some limitations. The number of dogs in each group was small,
362 and female dogs were overrepresented in the American cocker spaniel group. The health
363 status of the mesaticephalic dog group was based on retrospective data, hence it is possible
364 that some of these dogs may have suffered from otitis externa previously. The different
365 tympanic bulla measurements were performed by either one of the two observers, thus
366 reliability or repeatability could not be measured. Ideally, bulla measurements should have
367 been performed twice, but as some of the measurements were laborious, this was not
368 feasible.

369 In conclusion, the horizontal ear canal of healthy American cocker spaniels was
370 not narrower than that seen in similarly sized mesaticephalic dogs. The method of measuring
371 the transverse area of the horizontal ear canal appeared feasible with excellent intra- and
372 inter-observer repeatability. In American cocker spaniels, the mean tympanic bulla volume
373 was slightly smaller in relation to the body weight, and the tympanic bulla wall was thicker
374 than in previous reports for mesaticephalic dogs. Our results imply that breed-specific
375 differences in the anatomy of the bulla also appear in non-brachycephalic dog breeds.
376

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494 Table 1. Otoscopic and cytological examination findings of the ears in American cocker

495 spaniels. A total OTIS3 score of ≥ 4 differentiated affected from clinically healthy ears.

	Clinically healthy ears (n=40)	Clinically mild otitis externa (n=13)	Clinically severe otitis externa (n=22)
OTIS3 score for different parameters: mean ± SD (range)			
Erythema	0.3 ± 0.4 (0–1)	0.6 ± 0.5 (0–1)	1.2 ± 0.5 (1–3)
Edema	0.5 ± 0.6 (0–2)	0.9 ± 0.8 (0–2)	2.8 ± 0.4 (2–3)
Exudate	0.6 ± 0.5 (0–2)	2.5 ± 0.7 (1–3)	2.3 ± 0.7 (1–3)
Ulceration	0	0	0
Total	1.3 ± 1.2 (0–3)	4.2 ± 0.4 (4–5)	6.4 ± 0.6 (6–8)
Number of ears with the following cytological findings:			
Malassezia spp.	17	12	10
Cocci	1	1	14
Rods	0	1	16
Inflammatory cells	0	1	5

496

497 OTIS3 scoring: The presence and amount of erythema, edema, exudate and

498 erosion/ulceration in the ear canal were scored with 0-3 scale as follows: none (score 0),

499 mild (1), moderate (2), and marked (3) changes of the parameters.

500 SD: Standard Deviation.

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Table 2. Horizontal ear canal measurements and descriptive data of the healthy American cocker spaniels and mesaticephalic dogs in the study.

	American cocker spaniels (n = 9, 18 ears)	Mesaticephalic dogs (n = 23, 46 ears)	*P* -value
	Mean ± SD (range)	Mean ± SD (range)	
Weight (kg)	10.0 ± 0.9 (8.3–11.5)	10.4 ± 4.7 (5.2 – 19.5)	0.562
Age (years)	4.3 ± 2.2 (1.2–8.0)	7.1 ± 3.7 (1.8–13.0)	0.042*
Skull size (cm)	10.5 ± 0.4 (9.8–11.0)	10.4 ± 1.2 (8.2–12.7)	0.552
TA1 (mm^2)	27.2 ± 1.6 (24.6–30.1)	29.2 ± 7.0 (12.3–45.0)	0.522
TA2 (mm^2)	37.1 ± 7.8 (24.6–51.4)	35.6 ± 10.1 (16.9–59.4)	0.247

SD = Standard Deviation

TA1 = Transverse area (mm^2) of the osseous part of the horizontal ear canal

TA2 = Transverse area (mm^2) of the widest air-filled part of the horizontal ear canal

* = significant difference between the two groups

523 Table 3. Tympanic bulla measurements in American cocker spaniels.

	Group 1, n = 18			Group 2, n = 18			Group 3, n = 14			Group 4, n = 25		
	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD
Tympanic bulla wall, caudo-ventral, mm	0.68	2.67	1.26 ± 0.61	0.81	2.14	1.07 ± 0.31	0.73	3.84	1.30 ± 1.01	0.79	2.30	1.26 ± 0.46
Tympanic bulla wall, rostro-ventral, mm	0.85	2.58	1.55 ± 0.58	0.87	3.06	1.73 ± 0.78	0.26	3.26	1.57 ± 0.99	0.54	4.73	1.85 ± 0.96
Tympanic bulla volume, mm^3	0.50	1.07	0.77 ± 0.17	0.64	1.33	0.96 ± 0.21	0.65	1.09	0.83 ± 0.17	0.56	1.22	0.82 ± 0.17

524

525 SD: Standard Deviation

526 Group 1: Dogs with clinically healthy ears and no history of ear disease.

527 Group 2: Dogs with clinically healthy ears, and a history of ear disease.

528 Group 3: Dogs with clinically mild otitis externa.

529 Group 4: Dogs with clinically severe otitis externa.

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538 Figure legends:

539 **Figure 1A-C**: Multiplanar reconstruction images (middle-ear algorithm, window level 700 HU,
540 window width 4000 HU) showing the measurement point for measuring the transverse area
541 (mm^2) of the osseous (TA1) (B) and widest air-filled part of the cartilaginous (TA2) (C)
542 horizontal ear canal.

543 **Figure 2**: The transverse area (mm^2) of the horizontal ear canal was determined by manually
544 drawing a region of interest with the software's pencil tool and tracing the margin of the
545 bony wall of the osseous canal (A) or the air-filled cartilaginous canal (B).

546 **Figures 3**: The thickness of the tympanic bulla wall (mm) was measured in the transverse
547 plane images (middle-ear algorithm, window level 700 HU, window width 4000 HU) at the
548 level of the tympanic membrane (caudo-ventral aspect) (A) and at the level of the cochlea
549 (rostro-ventral aspect) (B).

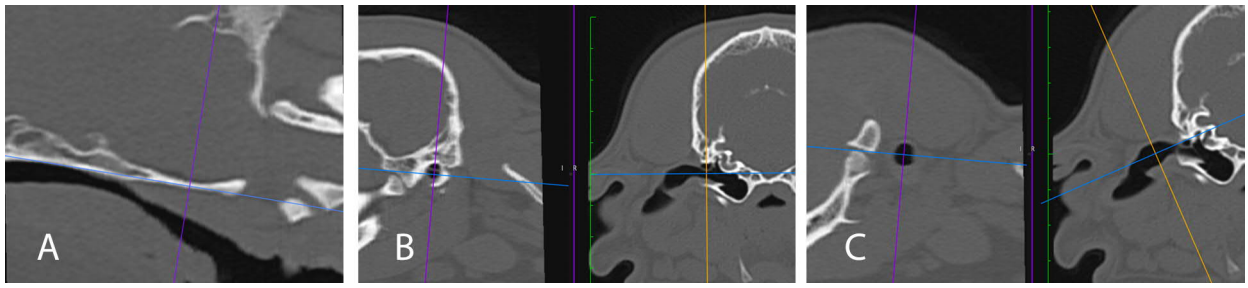
550 **Figure 4:** The skull width and height (cm) were measured from the transverse plane images
551 (middle-ear algorithm, window level 700 HU, window width 4000 HU) at the level of the
552 tympanic membrane as shown in the images.

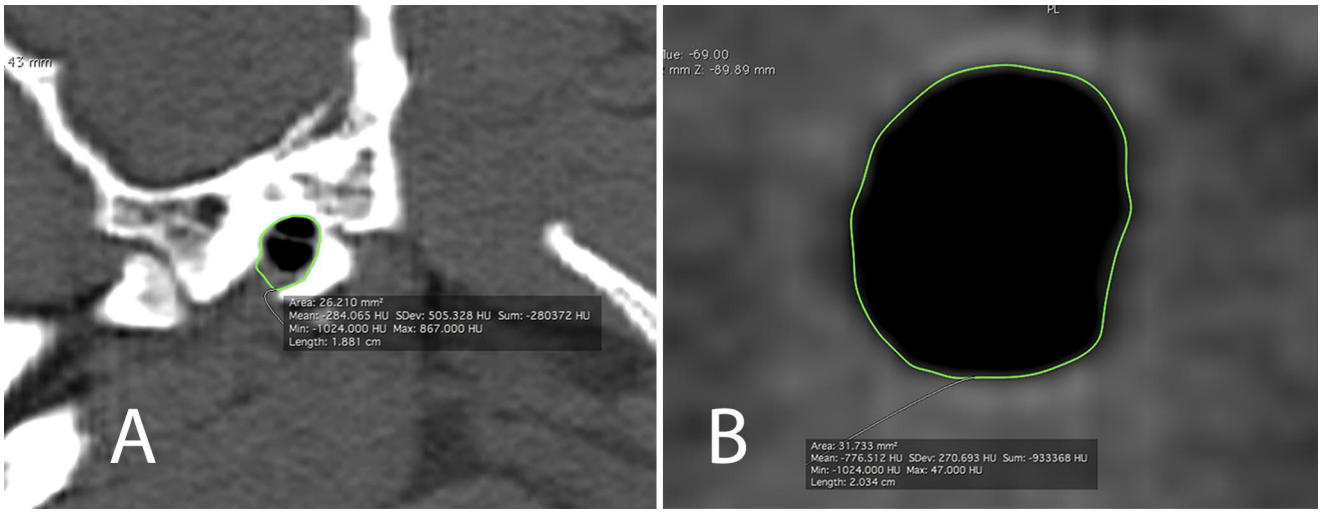
553 **Figure 5**: Comparison of the transverse area (mm^2) of the osseous part of the horizontal ear
554 canal (TA1) in different American cocker spaniel groups. No significant differences were
555 detected between the groups. Group 1: dogs with clinically healthy ears and no history of
556 ear disease. Group 2: dogs with clinically healthy ears, and a history of ear disease. Group 3:
557 dogs with clinically mild otitis externa. Group 4: dogs with clinically severe otitis externa. The
558 whiskers of the box plot show the minimum and maximum of the data, lower line of the box
559 presents the lower quartile, upper line the upper quartile and the line in the middle the
560 median. Outliers are presented with circles.

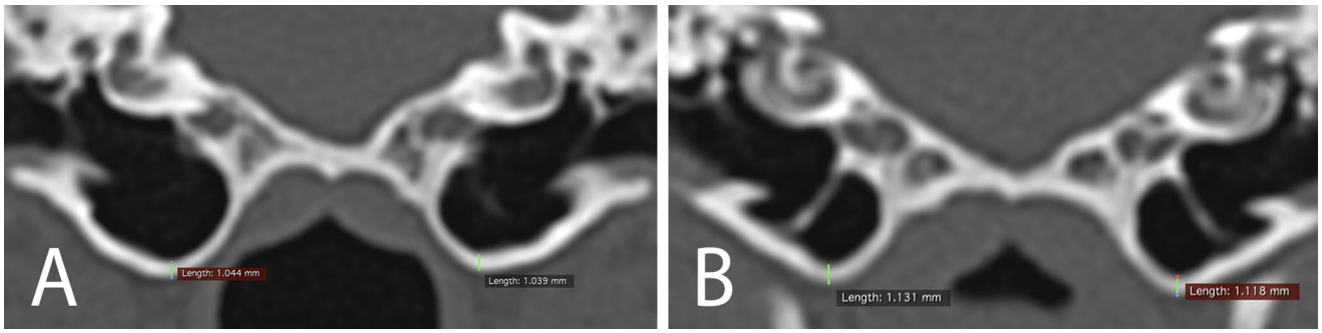
Figure 6: Comparison of the transverse area (mm^2) of the widest air-filled part of the cartilaginous horizontal ear canal (TA2) in different American cocker spaniel groups. The TA2 values in group 4 were significantly smaller than in the other groups. Group 1: Dogs with clinically healthy ears and no history of ear disease. Group 2: Dogs with clinically healthy ears, and a history of ear disease. Group 3: Dogs with clinically mild otitis externa. Group 4: Dogs with clinically severe otitis externa. The whiskers of the box plot show the minimum and maximum of the data, lower line of the box presents the lower quartile, upper line the upper quartile and the line in the middle the median. Outliers are presented with circles.

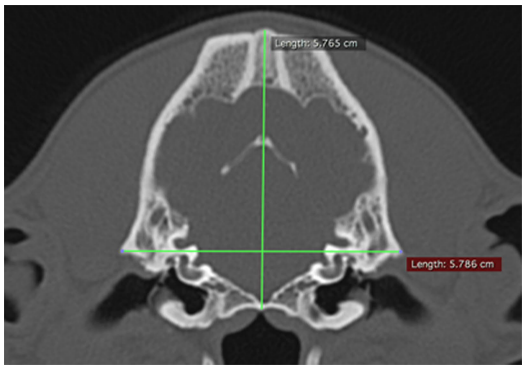
Figure 7: The thickness of the tympanic bulla wall varied between individuals. These images (slice thickness 1 mm, bone algorithm, window level 500 HU, window width 3500 HU) are from a clinically healthy American cocker spaniel with no history of ear disease, but with a thick tympanic bulla wall. The thickness of the tympanic bulla wall was measured at the level of the tympanic membrane (A) and at the level of the cochlea (B). Compare with Figure 3.

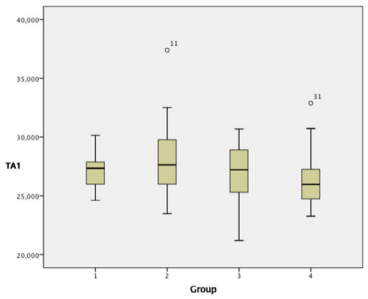
Figure 8: The Bland-Altman plot presents the difference between the calculated (MECV) and the measured tympanic bulla volume values in American cocker spaniels against the mean of these two. MECV = -0.612 + 0.757 * ln (bodyweight).

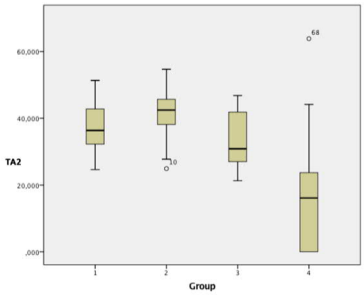

A
B
C

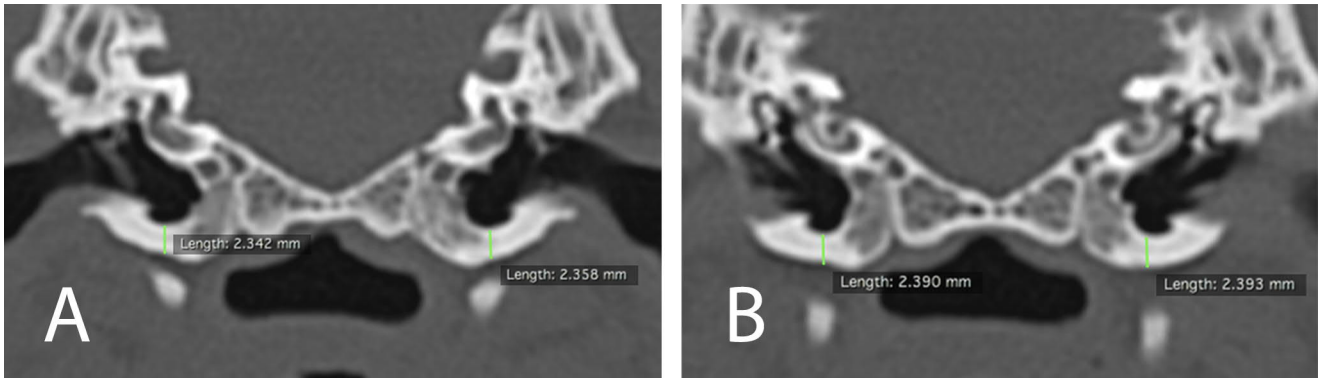

43 mm
Area: 26.210 mm²
Mean: -284.065 HU SDev: 505.328 HU Sum: -280372 HU
Min: -1024.000 HU Max: 867.000 HU
Length: 1.881 cm
A
ue: -69.00
mm Z: -89.89 mm
Area: 31.733 mm²
Mean: -776.512 HU SDev: 270.693 HU Sum: -933368 HU
Min: -1024.000 HU Max: 47.000 HU
Length: 2.034 cm
B

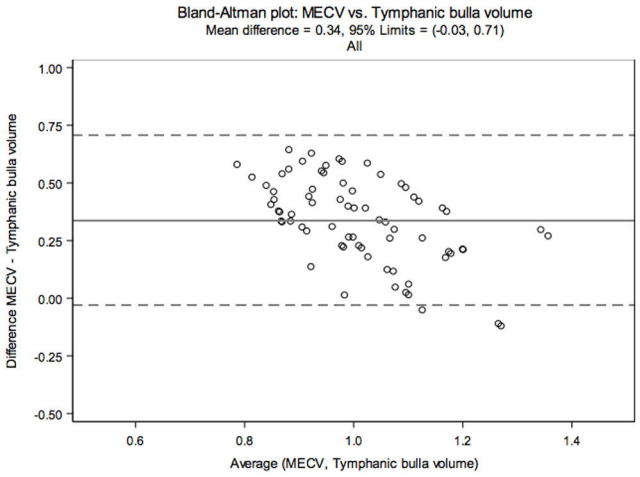

A
Length: 1.044 mm
Length: 1.039 mm
B
Length: 1.131 mm
Length: 1.118 mm


Length: 5.765 cm
Length: 5.786 cm


40,000
35,000
30,000
25,000
20,000
TA1
11
31
1
2
3
4
Group


60,000
40,000
20,000
,000
TA2
68
10
1
2
3
4
Group


Length: 2.342 mm
Length: 2.358 mm
A
Length: 2.390 mm
Length: 2.393 mm
B


Bland-Altman plot: MECV vs. Tymphanic bulla volume
Mean difference = 0.34, 95% Limits = (-0.03, 0.71)
All
Difference MECV - Tymphanic bulla volume
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
0.6
0.8
1.0
1.2
1.4
Average (MECV, Tymphanic bulla volume)